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(54) Title: SCREENING METHODS FOR COMPOUNDS USEFUL IN THE TREATMENT OF SCHIZOPHRENIA (57) Abstract The present invention relates to drug screening assays, and diagnostic and therapeutic methods for the treatment of schizophrenia utilizing the human neuron-specific glutamate transporter, called glutamate transporter III, as the target for intervention. The invention also relates to compounds that modulate the activity or expression of the transporter, and the use of such compounds, in the treatment of schizophrenia, and other disorders mediated by the transporter.		

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SCREENING METHODS FOR COMPOUNDS
USEFUL IN THE TREATMENT OF SCHIZOPHRENIA

1. INTRODUCTION

5 The present invention relates to screening assays for
compounds that modulate the activity of the neuron-specific
glutamate transporter (glutamate transporter III), and their
use in the treatment of schizophrenia and other neurological
disorders mediated by abnormal activity of glutamate
10 transporter III.

2. BACKGROUND OF THE INVENTION

In the mammalian central nervous system (CNS), glutamate
is released from nerve terminals during neurotransmission
15 serving as an excitatory neurotransmitter. Synaptic function
of glutamate is thought to be terminated by rapid uptake into
the surrounding glial cells or into the pre-synaptic nerve
terminals (Nicholls, D.G., 1993, Eur. J. Biochem. 212:613-
631). This high affinity uptake is accomplished by carrier
20 proteins, located in the plasma membrane of glutamatergic
nerve terminals and of glial cells (Nicholls, D.G., 1993,
Eur. J. Biochem. 212:613-631). The uptake does not
discriminate between aspartate or glutamate and depends on
the co-transport of Na⁺, and counter-transport of K⁺. Hence,
25 these proteins are known as Na⁺/K⁺ dependent
aspartate/glutamate transporters.

Four glutamate transporters have been reported. The
human aspartate/glutamate transporter III (Shashidharan et
al., 1994, Brain Research 662:245-250 which is incorporated
30 by reference herein in its entirety), is homologous to a
rabbit glutamate carrier (Kanai, Y., et al., 1992, Nature,
360:467-471) but it shows low homology to two human glutamate
transporters previously described (Shashidharan, P., et al.,
1993, Biochim. Biophys. Acta. 1216:161-164); (Shashidharan,
35 P., et al., 1994, Biochim. Biophys. Acta. 1191:393-396). As
with the rabbit carrier, (Kanai, Y., et al., 1992, Nature,
360:467-471) human aspartate/glutamate transporter III is
expressed in both neural and non-neural tissues. In the

central nervous system, this transporter mRNA is present in neurons and shows an extensive distribution pattern in cerebral cortical layers, substantia nigra, red nucleus and hippocampus. The glutamate transporter III is the only
5 glutamate transporter known to be expressed outside the central nervous system.

Previous observations suggested that glutamatergic pre-synaptic mechanisms are altered in neurodegenerative disorders, such as amyotrophic lateral sclerosis, and in
10 metabolic insults (ischemia, hypoglycemia, and thiamine deficiency) that lead to neuronal degeneration through excitotoxic mechanisms. However, the role of the glutamate transporters in various neurologic disorders is unknown.

15

3. SUMMARY OF THE INVENTION

The present invention relates to screening assays to identify compounds that modulate the activity of the glutamate transporter III (hereinafter "GTIII") and their use for the treatment of schizophrenia, and other neurological
20 disorders mediated by GTIII. The invention also relates to the compounds identified in the assays, diagnostic methods and treatment.

The invention is based, in part, on the discovery of a selective and significant reduction of the mRNA that encodes
25 the neuron-specific GTIII in the brains of schizophrenics, implicating a role of the transporter in the pathophysiology of schizophrenia. Thus, the compounds which modulate the activity of GTIII may be useful as antipsychotics for treating schizophrenia or other neurological disorders
30 mediated by GTIII, including but not limited to Parkinsons or Alzheimer's disease.

The invention relates to assays designed to screen for compounds or compositions that modulate GTIII activity. To this end, cell-based assays or non-cell based assays can be
35 used to detect compounds that interact with, e.g., bind to the GTIII. The cell-based assays have the advantage in that

they can be used to identify compounds that affect GTIII biological activity, i.e., glutamate transport.

The invention also relates to assays designed to screen for compounds or compositions that modulate GTIII gene expression. For example, cell-based assays, or cell-lysate assays (e.g., in vitro transcription or translation assays) can be used to screen for compounds or compositions that modulate GTIII transcription (e.g., compounds that modulate expression, production or activity of transcription factors involved in GTIII gene expression; polynucleotides that form triple helical structures with an GTIII regulatory region and inhibit transcription of the GTIII gene, etc.). Alternatively, cell-based assays or cell-lysate assays can be used to screen for compounds or compositions that modulate translation of GTIII transcripts (e.g., antisense and ribozyme molecules).

In yet another embodiment, the cell-based assays or cell-lysate assays can be used to test polynucleotide constructs designed to modify the expression of the GTIII gene in vivo. Such constructs include polynucleotide constructs designed for gene therapy; e.g., expression constructs or gene replacement constructs that place the GTIII gene under the control of a strong promoter system, an inducible promoter system or a constitutive promoter system.

The invention also encompasses agonists and antagonists of GTIII, including small molecules, large molecules, and antibodies, as well as nucleotide sequences that can be used to inhibit GTIII gene expression (e.g., antisense and ribozyme molecules), and gene or regulatory sequence replacement constructs designed to enhance GTIII gene expression (e.g., expression constructs that place the GTIII gene under the control of a strong promoter system). Such compounds may be used to treat schizophrenia, or other GTIII-mediated neuropathologies.

The invention also encompasses the use of such compounds and compositions, including gene therapy approaches, that

modulate GTIII activity or GTIII gene expression to treat schizophrenia, or other GTIII-mediated neuropathologies.

3.1. DEFINITIONS

5 The following terms as used herein shall have the meaning indicated.

GTIII nucleotides or coding sequences: means DNA sequences encoding GTIII mRNA transcripts, GTIII protein, polypeptide or peptide fragments of GTIII protein, or GTIII
10 fusion proteins. GTIII nucleotide sequences encompass DNA, including genomic DNA (e.g. the GTIII gene) or cDNA.

GTIII means GTIII gene products, e.g., transcripts and the GTIII protein. Polypeptides or peptide fragments of the GTIII protein are referred to as GTIII polypeptides or GTIII
15 peptides. Fusions of GTIII, or GTIII polypeptides, or peptide fragments to an unrelated protein are referred to herein as GTIII fusion proteins. A functional GTIII refers to a protein which transports glutamate into a cell.

20 ECD: means "extracellular domain".

TM: means "transmembrane domain".

CD: means "cytoplasmic domain".

4. DESCRIPTION OF THE FIGURES

25 FIG. 1. Nucleotide and deduced amino acid sequence of the human glutamate transporter III cDNA. Nucleotides corresponding to the homologous rabbit glutamate transporter (Kanai, Y., et al., 1992, Nature, 360:467-471) are shown in
italics directly above the human nucleotide sequence only where they are different. Also, predicted amino acids
30 corresponding to the rabbit cDNA (Kanai, Y., et al., 1992, Nature, 360:467-471) are also shown in italics directly below the human amino acid sequence only where they are different. Insertions are marked by relevant bases and deletions by
35 dashes. The nucleotide number is indicated on the left and the stop codon is indicated with an asterisk. The ten

putative transmembran regions are underlined; the potential N-glycosylation sites are indicated by double underlines.

5. DETAILED DESCRIPTION OF THE INVENTION

5 The invention encompasses screening methods (e.g., assays) for the identification of compounds which modulate the activity of GTIII. The invention also encompasses agonists and antagonists of GTIII, including small molecules, large molecules, and antibodies, as well as nucleotide
10 sequences that can be used to inhibit GTIII gene expression (e.g., antisense and ribozyme molecules), and gene or regulatory sequence replacement constructs designed to enhance GTIII gene expression (e.g., expression constructs that place the GTIII gene under the control of a strong
15 promoter system). Such compounds may be used to treat schizophrenics, or patients suffering from other neurological disorders mediated by GTIII.

 In particular, cellular and non-cellular assays are described that can be used to identify compounds that
20 interact with the GTIII, e.g., modulate the activity of the GTIII and/or bind to the GTIII. Such cell-based assays of the invention utilize cells, cell lines, or engineered cells that express the GTIII; including genetically engineered host cells that express GTIII, or GABAergic cells or cell lines.

25 The invention also encompasses the use of cell-based assays or cell-lysate assays (e.g., in vitro transcription or translation assays) to screen for compounds or compositions that modulate GTIII gene expression. To this end, constructs containing a reporter sequence linked to a regulatory element
30 of the GTIII gene can be used in engineered cells, or in cell lysate extracts, to screen for compounds that modulate the expression of the reporter gene product at the level of transcription. For example, such assays could be used to identify compounds that modulate the expression or activity
35 of transcription factors involved in GTIII gene expression, or to test the activity of triple helix polynucleotides.

Alternatively, engineered cells or translation extracts can be used to screen for compounds (including antisens and ribozyme constructs) that modulate the translation of GTIII mRNA transcripts, and therefore, affect expression of the
5 GTIII.

The invention also encompasses GTIII proteins, polypeptides (including soluble GTIII polypeptides or peptides), GTIII fusion proteins, and peptides (including synthetic peptides) derived from the GTIII amino acid
10 sequence for use in non-cell based screening assays, for use in generating antibodies, for diagnostics and therapeutics. The GTIII is predicted to traverse the cell membrane ten times (see FIG. 1). Thus, the domains located between the transmembrane domain contribute to the extracellular domain
15 and cytoplasmic domain of GTIII. Peptides corresponding to each domain or polypeptides composed of two or more of the domains linked together can be engineered as described in Section 5.4.1, infra. Such peptides or polypeptides can be fused to a heterologous protein, e.g., a reporter, an Ig Fc
20 region, etc., to yield a fusion protein. Such peptides, polypeptides and fusion proteins may be used in the non-cell based assays for screening compounds that interact with or bind to the GTIII.

The invention also encompasses diagnostic assays for
25 schizophrenia, or other disorders mediated by GTIII, or other disorders mediated by GTIII, using hybridization analyses to detect mutations in a patient's GTIII gene, or the level of transcription of GTIII protein in patient samples. Analysis of genomic DNA for mutations in the patient's GTIII gene can
30 be performed on any cell sample taken from the patient, e.g., blood samples, skin, bone marrow, etc. The patient material used to analyze transcription levels of GTIII should be obtained from tissues that express the GTIII gene, e.g., hippocampus, brain, neuronal tissue samples. Alternatively
35 antibodies to GTIII can be used to detect levels of expression of GTIII protein in patient samples, or in situ using imaging techniques; e.g., labeled antibody coupled with

appropriate imaging procedures such as CAT scans, NMR etc. The detection of a defective GTIII gene, reduced transcripts and/or reduced protein levels would indicate schizophrenia or another GTIII-mediated neurological disorder.

- 5 "Gene therapy" approaches for the modulation of GTIII expression and/or activity in the treatment of schizophrenia or other disorders mediated by GTIII are also within the scope of the invention. For example, nucleotide constructs encoding functional GTIIIs, mutant GTIIIs, as well as
10 antisense and ribozyme molecules can be used to modulate GTIII expression.

The invention also encompasses pharmaceutical formulations and methods for treatment.

15 5.1. THE ROLE OF GTIII IN SCHIZOPHRENIA

- The specific role of GTIII in schizophrenia was investigated by studying the expression of GTIII in the brains of schizophrenics vs. normal controls. The levels of GTIII expressed in neural tissue was investigated using both
20 RNAase protection and *in situ* hybridization. For both methods, a Taq 1 fragment of the GTIII cDNA, *i.e.*, nucleotide 1065-1319 of FIG. 1 was used as a probe.

- To evaluate alterations in GTIII expression, levels of GTIII mRNA were measured using quantitative nuclease
25 protection assays in hippocampus homogenates of schizophrenics (n=19), suicides (n=15) and normal controls (n=19). A 50% decrease in GTIII mRNA was observed in schizophrenics (p=0.0005) and a 20% decrease in suicide (p=0.1227) compared to normal controls.

- 30 In addition, *in situ* hybridization histochemistry was performed with an ³⁵S-labelled ribonucleotide probe using frozen sections containing parahippocampal gyrus, hippocampus, presubiculum and subiculum tissue from schizophrenics (n=9) and normal controls (n=7). A two-way
35 ANOVA (group by region) indicated that there was a reduction in GTIII mRNA in schizophrenics (p<.0001) when compared to

normal controls. Comparisons for each region indicated that the difference was statistically significant ($p \leq .05$) in dentate, CA1 and subiculum where levels were 69, 67 and 61% respectively, of normal levels. Decreased levels were also observed ($.08 \geq p > .05$) in CA4, presubiculum and parahippocampal layers ii/iii.

Both the RNA protection studies and the *in situ* hybridization studies indicate that aberrant hippocampal expression of GTIII plays a role in the neuropathology of schizophrenia.

5.2. SCREENING ASSAYS FOR COMPOUNDS THAT MODULATE GTIII

At least three different assay systems, described in the subsections below, can be designed and used to identify compounds or compositions that modulate GTIII activity or GTIII gene expression.

The systems described below may be formulated into kits. To this end, GTIII or cells expressing GTIII can be packaged in a variety of containers, e.g., vials, tubes, microtitre well plates, bottles, and the like. Other reagents can be included in separate containers and provided with the kit; e.g., positive controls samples, negative control samples, buffers, cell culture media, etc.

5.2.1. CELL-BASED ASSAYS

In accordance with the invention, a cell-based assay system can be used to screen for compounds that modulate the activity of the GTIII, and, therefore, may be useful to treat schizophrenia and other neurological disorders mediated by GTIII. Such disorders may include Parkinsons and Alzheimer's. To this end, cells that endogenously express GTIII can be used in the assay, e.g., GABAergic cells such as Purkinje cells of the cerebellum. Alternatively, cell lines, such as 293 cells, COS cells, CHO cells, fibroblasts, and the like, genetically engineered to express GTIII can be used for

screening purposes. Preferably, host cells genetically engineered to express a functional GTIII that transports glutamate should be used. Glutamate transport can be measured using standard methods known to those skilled in the art to assay for glutamate uptake by the host cell. For example, cellular uptake of labeled glutamate from the culture media can be measured. Alternatively, when GABAergic cells are used, host cell production of GABA can be measured (glutamate taken up by such cells is a precursor for the synthesis of GABA).

In utilizing such cell systems, the cells expressing GTIII are exposed to a test compound or to vehicle controls (e.g., placebos). After exposure, the cells can be assayed to measure glutamate transport. The ability of a test compound to increase levels of transport, above those levels seen with cells treated with a vehicle control, indicates that the test compound enhances the activity of GTIII expressed by the host cell.

20 5.2.2. NON-CELL BASED ASSAYS

In addition to cell based assays, non-cell based assay systems may be used to identify compounds that interact with, e.g., bind to GTIII. Such compounds may act as antagonists or agonists of GTIII activity.

25 Isolated membranes may be used to identify compounds that interact with GTIII. For example, in a typical experiment using isolated membranes, 293 cells may be genetically engineered to express the GTIII. Membranes can be harvested by standard techniques and used in an in vitro binding assay, e.g., labeled test compound.

30 Alternatively, soluble GTIII may be recombinantly expressed and utilized in non-cell based assays to identify compounds that bind to GTIII. Recombinantly expressed GTIII polypeptides, peptides, or fusion proteins containing one or more of the domains that contribute to the ECD prepared as described in Section 5.4.1, infra, can be used in the non-

cell based screening assays. Alternatively, peptides corresponding to the domains that contribute to the CD of GTIII, or fusion proteins containing one or more of these peptides can be used in non-cell based assay systems to
5 identify compounds that bind to the cytoplasmic portion of the GTIII; such compounds may be useful to modulate the activity of GTIII. In non-cell based assays the recombinantly expressed GTIII can be conveniently attached to a solid substrate such as a test tube, microtitre well or a
10 column, by means well known to those in the art (see Ausubel et al., supra). The test compounds are then assayed for their ability to bind to the GTIII.

15 5.2.3. ASSAYS FOR COMPOUNDS OR COMPOSITIONS
THAT MODULATE EXPRESSION OF GTIII

In vitro cell based assays may be designed to screen for compounds that regulate GTIII expression at either the transcriptional or translational level.

In one embodiment, DNA encoding a reporter molecule can
20 be linked to a regulatory element of the GTIII gene and used in appropriate intact cells, cell extracts or lysates to identify compounds that modulate GTIII gene expression. Appropriate cells or cell extracts are prepared from any cell type that normally expresses the GTIII gene, thereby ensuring
25 that the cell extracts contain the transcription factors required for in vitro or in vivo transcription. The screen can be used to identify compounds that modulate the expression of the reporter construct. In such screens, the level of reporter gene expression is determined in the
30 presence of the test compound and compared to the level of expression in the absence of the test compound.

To identify compounds that regulate GTIII translation, cells or in vitro cell lysates containing GTIII transcripts may be tested for modulation of GTIII mRNA translation. To
35 assay for inhibitors of GTIII translation, test compounds are assayed for their ability to modulate the translation of GTIII mRNA in in vitro translation extracts.

Compounds that increase the level of GTIII expression, either at the transcriptional or translational level, may be useful for treatment of schizophrenia.

5 5.2.4. COMPOUNDS THAT CAN BE SCREENED
 IN ACCORDANCE WITH THE INVENTION

The compounds which may be screened in accordance with the invention include, but are not limited to peptides, antibodies and fragments thereof, and other organic compounds
10 (e.g., peptidomimetics) that bind to GTIII and either enhance the activity of GTIII as well as peptides, antibodies or fragments thereof, and other organic compounds.

Compounds may include, but are not limited to, peptides such as, for example, soluble peptides, including but not
15 limited to members of random peptide libraries; (see, e.g., Lam, K.S. et al., 1991, Nature 354:82-84; Houghten, R. et al., 1991, Nature 354:84-86), and combinatorial chemistry-derived molecular library made of D- and/or L- configuration amino acids, phosphopeptides (including, but not limited to,
20 members of random or partially degenerate, directed phosphopeptide libraries; see, e.g., Songyang, Z. et al., 1993, Cell 72:767-778), antibodies (including, but not limited to, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain antibodies, and FAb,
25 F(ab')₂, and FAb expression library fragments, and epitope-binding fragments thereof), and small organic or inorganic molecules.

Other compounds which can be screened in accordance with the invention include but are not limited to small organic
30 molecules that are able to cross the blood-brain barrier, gain entry into an appropriate cell and affect the expression of the GTIII gene; or such compounds that affect the activity of the GTIII or the activity.

Computer modelling and searching technologies permit
35 identification of compounds, or the improvement of already identified compounds, that can modulate GTIII expression or activity. Having identified such a compound or composition,

the active sites or regions are identified. Such active sites might typically be ligand binding sites. The active site can be identified using methods known in the art including, for example, from the amino acid sequences of 5 peptides, from the nucleotide sequences of nucleic acids, or from study of complexes of the relevant compound or composition with its natural ligand. In the latter case, chemical or X-ray crystallographic methods can be used to find the active site by finding where on the factor the 10 complexed ligand is found.

Next, the three dimensional geometric structure of the active site is determined. This can be done by known methods, including X-ray crystallography, which can determine a complete molecular structure. On the other hand, solid or 15 liquid phase NMR can be used to determine certain intramolecular distances. Any other experimental method of structure determination can be used to obtain partial or complete geometric structures. The geometric structures may be measured with a complexed ligand, natural or artificial, 20 which may increase the accuracy of the active site structure determined.

If an incomplete or insufficiently accurate structure is determined, the methods of computer based numerical modelling can be used to complete the structure or improve its 25 accuracy. Any recognized modelling method may be used, including parameterized models specific to particular biopolymers such as proteins or nucleic acids, molecular dynamics models based on computing molecular motions, statistical mechanics models based on thermal ensembles, or 30 combined models. For most types of models, standard molecular force fields, representing the forces between constituent atoms and groups, are necessary, and can be selected from force fields known in physical chemistry. The incomplete or less accurate experimental structures can serve 35 as constraints on the complete and more accurate structures computed by these modelling methods.

Finally, having determined the structure of the active site, either experimentally, by modeling, or by a combination, candidate modulating compounds can be identified by searching databases containing compounds along with
5 information on their molecular structure. Such a search seeks compounds having structures that match the determined active site structure and that interact with the groups defining the active site. Such a search can be manual, but is preferably computer assisted. These compounds found from this search
10 are potential GTIII modulating compounds.

Alternatively, these methods can be used to identify improved modulating compounds from an already known modulating compound or ligand. The composition of the known compound can be modified and the structural effects of
15 modification can be determined using the experimental and computer modelling methods described above applied to the new composition. The altered structure is then compared to the active site structure of the compound to determine if an improved fit or interaction results. In this manner
20 systematic variations in composition, such as by varying side groups, can be quickly evaluated to obtain modified modulating compounds or ligands of improved specificity or activity.

Further experimental and computer modeling methods
25 useful to identify modulating compounds based upon identification of the active sites of GTIII, and related transduction and transcription factors will be apparent to those of skill in the art.

Examples of molecular modelling systems are the CHARMM
30 and QUANTA programs (Polygen Corporation, Waltham, MA). CHARMM performs the energy minimization and molecular dynamics functions. QUANTA performs the construction, graphic modelling and analysis of molecular structure. QUANTA allows interactive construction, modification,
35 visualization, and analysis of the behavior of molecules with each other.

A number of articles review computer modelling of drugs interactive with specific proteins, such as Rotivinen, et al.) 1988, Acta Pharmaceutical Fennica 97:159-166); Ripka (1988 New Scientist 54-57); McKinaly and Rossmann (1989, 5 Annu. Rev. Pharmacol. Toxicol. 29:111-122); Perry and Davies, O²AR: Quantitative Structure-Activity Relationships in Drug Design pp. 189-193 Alan R. Liss, Inc. 1989; Lewis and Dean (1989, Proc. R. Soc. Lond. 236:125-140 and 141-162); and, with respect to a model receptor for nucleic acid components, Askew, et al. (1989, J. Am. Chem. Soc. 111:1082-1090). Other computer programs that screen and graphically depict chemicals are available from companies such as BioDesign, Inc. (Pasadena, CA.), Allelix, Inc. (Mississauga, Ontario, Canada), and Hypercube, Inc. (Cambridge, Ontario). 15 Although these are primarily designed for application to drugs specific to particular proteins, they can be adapted to design of drugs specific to regions of DNA or RNA, once that region is identified.

Although described above with reference to design and 20 generation of compounds which could alter binding, one could also screen libraries of known compounds, including natural products or synthetic chemicals, and biologically active materials, including proteins, for compounds which are inhibitors or activators.

25

5.3. ANIMAL MODELS

Compounds identified via assays such as those described herein may be useful, for example, in elaborating the biological function of the GTIII gene product, and for 30 ameliorating schizophrenia, or other GTIII-mediated disorders. Assays for testing the efficacy of compounds identified in the cellular screen can be tested in animal model systems. Such animal models may be used as test substrates for the identification of drugs, pharmaceuticals, 35 therapies and interventions which may be effective in treating schizophrenia and other neurological disorders. For

example, animal models may be exposed to a compound. The response of the animals to the exposure may be monitored by assessing the level of glutamate in the nervous tissue of the animals, or the plasma concentration of glutamate. Compounds that stimulate glutamate transport (and reduce plasma concentration and glutamate accumulation in the nervous tissue) should be useful for treating schizophrenia. Dosages of test agents may be determined by deriving dose-response curves, as discussed in Section 5.6, below.

10

5.3.1. TRANSGENIC ANIMALS

Transgenic animals that express the human GTIII gene products, or defective GTIII can be used as model systems. Animals of any species, including, but not limited to, mice, rats, rabbits, guinea pigs, pigs, micro-pigs, goats, and non-human primates, e.g., baboons, monkeys, and chimpanzees may be used to generate GTIII transgenic animals.

Any technique known in the art may be used to introduce the human GTIII transgene into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to pronuclear microinjection (Hoppe, P.C. and Wagner, 1989, U.S. Pat. No. 4,873,191); retrovirus mediated gene transfer into germ lines (Van der Putten et al., 1985, Proc. Natl. Acad. Sci., USA 82:6148-6152); gene targeting in embryonic stem cells (Thompson et al., 1989, Cell 56:313-321); electroporation of embryos (Lo, 1983, Mol Cell. Biol. 3:1803-1814); and sperm-mediated gene transfer (Lavitrano et al., 1989, Cell 57:717-723); etc. For a review of such techniques, see Gordon, 1989, Transgenic Animals, Intl. Rev. Cytol. 115:171-229, which is incorporated by reference herein in its entirety.

The present invention provides for transgenic animals that carry the GTIII transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, i.e., mosaic animals. The transgene may be integrated as a single transgene or in concatamers, e.g., head-to-head tandems or head-to-tail tandems. The transgene may also be

selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko, M. et al., 1992, Proc. Natl. Acad. Sci. USA 89: 6232-6236). The regulatory sequences required for such a
5 cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. When it is desired that the GTIII transgene be integrated into the chromosomal site of the endogenous GTIII gene, gene targeting is preferred. Briefly, when such a
10 technique is to be utilized, vectors containing nucleotide sequences homologous to the endogenous GTIII gene and/or sequences flanking the gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the endogenous
15 GTIII gene. The transgene may also be selectively expressed in a particular cell type with concomitant inactivation of the endogenous GTIII gene in only that cell type, by following, for example, the teaching of Gu et al. (Gu, et al., 1994, Science 265: 103-106). The regulatory sequences
20 required for such a cell-type specific recombination will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

Once founder animals have been generated, standard techniques such as Southern blot analysis or PCR techniques
25 are used to analyze animal tissues to determine whether integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the founder animals may also be assessed using techniques which include but are not limited to Northern blot analysis of
30 tissue samples obtained from the animal, in situ hybridization analysis, and RT-PCR. Samples of the GTIII gene-expressing tissue, may also be evaluated immunocytochemically using antibodies specific for the GTIII transgene product.

35

5.3.2. INHIBITION OF GTIII EXPRESSION AND GENERATION OF ANIMAL MODELS

In an alternate embodiment of the invention, animal models for schizophrenia, or other GTIII-mediated neurological disorders can be designed by reducing the level of endogenous GTIII expression, e.g., using antisense or ribozyme approaches to inhibit or prevent translation of GTIII mRNA transcripts; triple helix approaches to inhibit transcription of the GTIII gene; or targeted homologous recombination to inactivate or "knock out" the GTIII gene or its endogenous promoter. Because the GTIII gene is expressed in the brain, delivery techniques should be preferably designed to cross the blood-brain barrier (see PCT WO89/10134, which is incorporated by reference herein in its entirety). Alternatively, the antisense, ribozyme or DNA constructs described herein could be administered directly to the site containing the target cells.

Antisense approaches involve the design of oligonucleotides (either DNA or RNA) that are complementary to mRNA. The antisense oligonucleotides will bind to the complementary mRNA transcripts and prevent translation. Absolute complementarity, although preferred, is not required. A sequence "complementary" to a portion of an RNA, as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

While antisense nucleotides complementary to the coding region sequence could be used, those complementary to the transcribed untranslated region are most preferred. Oligonucleotides that are complementary to the 5' end of the message, e.g., the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have recently shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., 1994, Nature 372:333-335. Thus, oligonucleotides complementary to either the 5'- or 3'- non-translated, non-coding regions of GTIII could be used in an antisense approach to inhibit translation of endogenous mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5'- , 3'- or coding region of GTIII mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

Regardless of the choice of target sequence, it is preferred that *in vitro* studies are first performed to quantitate the ability of the antisense oligonucleotide to inhibit gene expression. It is preferred that these studies utilize controls that distinguish between antisense gene inhibition and nonspecific biological effects of oligonucleotides. It is also preferred that these studies compare levels of the target RNA or protein with that of an internal control RNA or protein. Additionally, it is envisioned that results obtained using the antisense oligonucleotide are compared with those obtained using a

control oligonucleotide. It is preferred that the control oligonucleotide is of approximately the same length as the test oligonucleotide and that the nucleotide sequence of the oligonucleotide differs from the antisense sequence no more
5 than is necessary to prevent specific hybridization to the target sequence.

The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be
10 modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors in vivo), or agents facilitating transport
15 across the cell membrane (see, e.g., Letsinger et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556; Lemaitre et al., 1987, Proc. Natl. Acad. Sci. 84:648-652; PCT Publication No. WO88/09810, published December 15, 1988) or the blood-brain barrier (see, e.g., PCT Publication No. WO89/10134, published
20 April 25, 1988), hybridization-triggered cleavage agents. (See, e.g., Krol et al., 1988, BioTechniques 6:958-976) or intercalating agents. (See, e.g., Zon, 1988, Pharm. Res. 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a peptide,
25 hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including but not limited to 5-fluorouracil, 5-bromouracil,
30 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine,
35 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine,

5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, 5 queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

10 The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including but not limited to arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense oligonucleotide 15 comprises at least one modified phosphate backbone selected from the group consisting of a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

20 In yet another embodiment, the antisense oligonucleotide is an α -anomeric oligonucleotide. An α -anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gautier et al., 1987, 25 Nucl. Acids Res. 15:6625-6641). The oligonucleotide is a 2'-O-methylribonucleotide (Inoue et al., 1987, Nucl. Acids Res. 15:6131-6148), or a chimeric RNA-DNA analogue (Inoue et al., 1987, FEBS Lett. 215:327-330).

Oligonucleotides of the invention may be synthesized by 30 standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (1988, Nucl. Acids Res. 16:3209), 35 methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451), etc.

The antisense molecules should be delivered to cells which express GTIII in vivo, e.g., neural tissue. A number of methods have been developed for delivering antisense DNA or RNA to cells; e.g., antisense molecules can be injected directly into the tissue site, or modified antisense molecules, designed to target the desired cells (e.g., antisense linked to peptides or antibodies that specifically bind receptors or antigens expressed on the target cell surface) can be administered systemically.

10 However, it is often difficult to achieve intracellular concentrations of the antisense sufficient to suppress translation of endogenous mRNAs. Therefore a preferred approach utilizes a recombinant DNA construct in which the antisense oligonucleotide is placed under the control of a strong pol III or pol II promoter. The use of such a construct to transfect target cells in the patient will result in the transcription of sufficient amounts of single stranded RNAs that will form complementary base pairs with the endogenous GTIII transcripts and thereby prevent translation of the GTIII mRNA. For example, a vector can be introduced in vivo such that it is taken up by a cell and directs the transcription of an antisense RNA. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the antisense RNA can be by any promoter known in the art to act in mammalian, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include but are not limited to: the SV40 early promoter region (Bernt and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-

- 1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42), etc. Any type of plasmid, cosmid, YAC or viral vector can be used to prepare the recombinant DNA construct which can be introduced
- 5 directly into the tissue site; e.g., the hippocampus. Alternatively, viral vectors can be used which selectively infect the desired tissue; (e.g., for brain, herpesvirus vectors may be used), in which case administration may be accomplished by another route (e.g., systemically).
- 10 Ribozyme molecules designed to catalytically cleave GTIII mRNA transcripts can also be used to prevent translation of GTIII mRNA and expression of GTIII. (See, e.g., PCT International Publication WO90/11364, published October 4, 1990; Sarver et al., 1990, Science 247:1222-1225).
- 15 While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy GTIII mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole
- 20 requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, 1988, Nature, 334:585-591. Preferably the ribozyme is engineered
- 25 so that the cleavage recognition site is located near the 5' end of the GTIII mRNA; i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

- The ribozymes of the present invention also include RNA
- 30 endoribonucleases (hereinafter "Cech-type ribozymes") such as the one which occurs naturally in Tetrahymena Thermophila (known as the IVS, or L-19 IVS RNA) and which has been extensively described by Thomas Cech and collaborators (Zaug, et al., 1984, Science, 224:574-578; Zaug and Cech, 1986,
- 35 Science, 231:470-475; Zaug, et al., 1986, Nature, 324:429-433; published International patent application No. WO 88/04300 by University Patents Inc.; Been and Cech, 1986,

Cell, 47:207-216). The Cech-type ribozymes have an eight base pair active site which hybridizes to a target RNA sequenc whereafter cleavag of the target RNA takes place. The invention encompasses those Cech-type ribozymes which
5 target eight base-pair active site sequences that are present in GTIII mRNA.

As in the antisense approach, the ribozymes can be composed of modified oligonucleotides (e.g. for improved stability, targeting, etc.) and should be delivered to cells
10 which express the GTIII in vivo, e.g., hippocampus. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to
15 destroy endogenous GTIII messages and inhibit translation. Because ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Endogenous GTIII gene expression can also be reduced by
20 inactivating or "knocking out" the GTIII gene or its promoter using targeted homologous recombination (e.g., see Smithies et al., 1985, Nature 317:230-234; Thomas & Capecchi, 1987, Cell 51:503-512; Thompson et al., 1989 Cell 5:313-321; each of which is incorporated by reference herein in its
25 entirety). For example, a mutant, non-functional GTIII (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous GTIII gene can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express GTIII in vivo. Insertion of the
30 DNA construct, via targeted homologous recombination, results in inactivation of the GTIII gene. Such approaches are particularly suited in the agricultural field where modifications to ES (embryonic stem) cells can be used to generate animal offspring with an inactive GTIII (e.g., see
35 Thomas & Capecchi 1987 and Thompson 1989, supra).

Alternatively, endogenous GTIII gene expression can b reduced by targeting deoxyribonucleotide sequences

complementary to the regulatory region of the GTIII gene (i.e., the GTIII promoter and/or enhancers) to form triple helical structures that prevent transcription of the GTIII gene in target cells in the body. (See generally, Helene, C. 5 1991, Anticancer Drug Des., 6(6):569-84; Helene, C., et al., 1992, Ann, N.Y. Acad. Sci., 660:27-36; and Maher, L.J., 1992, Bioassays 14(12):807-15).

5.4. GTIII PROTEINS, POLYPEPTIDES, AND ANTIBODIES

10 GTIII protein, polypeptides and peptide fragments, mutated, truncated or deleted forms of the GTIII and/or GTIII fusion proteins can be prepared for a variety of uses, including but not limited to the generation of antibodies, as reagents in diagnostic assays, as reagents in assays for 15 screening for compounds that can be used in the treatment of schizophrenia and other disorders mediated by GTIII, and as pharmaceuticals useful in the treatment of schizophrenia or other disorders mediated by GTIII.

20 5.4.1. PRODUCTION OF GTIII POLYPEPTIDES

The deduced amino acid sequences of the human GTIII is shown in FIG. 1, where the predicted ten transmembrane domains are underlined. The hydrophilic domains located between the TM domains contribute to the structure of ECD and 25 the CD of the transporter. Peptides corresponding to one or more domains of the GTIII (e.g., ECD, TM or CD), truncated or deleted GTIII (e.g., GTIII in which one or more of the domains of the ECD, TM and/or CD is deleted) as well as fusion proteins in which the full length GTIII, a GTIII 30 peptide or truncated GTIII is fused to an unrelated protein are also within the scope of the invention. To this end, peptides corresponding to individual domains located between the ten transmembrane domains, or a polypeptide consisting of each of these domains linked together can be fused to another 35 polypeptide (e.g., an IgFc polypeptide). Alternatively, mutant GTIII proteins or polypeptides can be prepared; such mutants include but are not limited to GTIII in which the

glycosylation sites are eliminated to improve production and yield.

Such peptides, polypeptides, and fusion proteins can be prepared by recombinant DNA techniques. For example, nucleotide sequences encoding one or more of the domains that occur between the transmembrane domains cloned and ligated together to encode a soluble molecule. The DNA sequences encoding one or more of these domains can be ligated together directly or via a linker oligonucleotide that encodes a peptide spacer. Such linkers may encode flexible, glycine-rich amino acid sequences thereby allowing the domains that are strung together to assume a conformation that corresponds to their native conformation. Alternatively, nucleotide sequences encoding individual domains within the ECD can be used to express GTIII peptides.

A variety of host-expression vector systems may be utilized to express nucleotide sequences encoding the appropriate regions of the GTIII to produce such polypeptides. Where the resulting peptide or polypeptide is a soluble derivative (e.g., peptides corresponding to the domains located between the TMs, or truncated or deleted GTIII in which the TMs and/or CDs are deleted) the peptide or polypeptide can be recovered from the culture media. Where the polypeptide or protein is not secreted, the GTIII product can be recovered from the host cell itself.

The host-expression vector systems also encompass engineered host cells that express the GTIII or functional equivalents in situ, i.e., anchored in the cell membrane. Purification or enrichment of the GTIII from such expression systems can be accomplished using appropriate detergents and lipid micelles and methods well known to those skilled in the art. However, such engineered host cells themselves may be used in situations where it is important not only to retain the structural and functional characteristics of the GTIII, but to assess biological activity, e.g., in drug screening assays.

The host-expression vector systems that may be used for purposes of the invention include but are not limited to microorganisms such as bacteria (e.g., E. coli, B. subtilis) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing GTIII nucleotide sequences; yeast (e.g., Saccharomyces, Pichia) transformed with recombinant yeast expression vectors containing the GTIII nucleotide sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing the GTIII sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing GTIII nucleotide sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the GTIII gene product being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of GTIII protein or for raising antibodies to the GTIII protein, for example, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the E. coli expression vector pUR278 (Ruther et al., 1983, EMBO J. 2:1791), in which the GTIII coding sequence may be ligated individually into the vector in frame with the lacZ coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, Nucleic Acids Res. 13:3101-3109; Van Heeke & Schuster, 1989, J. Biol. Chem. 264:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione

S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The PGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

Alternatively, any fusion protein may be readily purified by utilizing an antibody specific for the fusion protein being expressed. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht, et al., 1991, Proc. Natl. Acad. Sci. USA 88: 8972-8976). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the gene's open reading frame is translationally fused to an amino-terminal tag consisting of six histidine residues. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni²⁺-nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

In an insect system, Autographa californica nuclear polyhidrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in Spodoptera frugiperda cells. The GTIII coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of GTIII coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (i.e., virus lacking the proteinaceous coat coded for by the polyhedrin gene). The recombinant viruses are then used to infect cells in which the inserted gene is expressed. (E.g., see Smith et al., 1983, J. Virol. 46: 584; Smith, U.S. Patent No. 4,215,051).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an

ad novirus is used as an expression vector, the GTIII nucleotide sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the GTIII gene product in infected hosts.

(E.g., See Logan & Shenk, 1984, Proc. Natl. Acad. Sci. USA 81:3655-3659). Specific initiation signals may also be required for efficient translation of inserted GTIII nucleotide sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where an entire GTIII gene or cDNA, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only a portion of the GTIII coding sequence is inserted, exogenous translational control signals, including, perhaps, the ATG initiation codon, must be provided. Furthermore, the initiation codon must be in frame with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (See Bittner et al., 1987, Methods in Enzymol. 153:516-544).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins

and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. Accordingly, eukaryotic host cells which possess the cellular machinery 5 for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include, but are not limited to, CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3 and WI38 cell lines.

10 For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the GTIII sequences described above may be engineered. Rather than using expression vectors which contain viral origins of replication, host 15 cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow 20 for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded 25 into cell lines. This method may advantageously be used to engineer cell lines which express the GTIII gene product. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that affect the endogenous activity of the GTIII gene product.

30 A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler, et al., 1977, Cell 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 1962, Proc. Natl. Acad. Sci. USA 48:2026), and adenine 35 phosphoribosyltransferase (Lowy, et al., 1980, Cell 22:817) genes can be employed in tk⁻, hgp^rt⁻ or ap^rt⁻ cells, respectively. Also, antimetabolite resistance can be used as

the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler, et al., 1980, Natl. Acad. Sci. USA 77:3567; O'Hare, et al., 1981, Proc. Natl. Acad. Sci. USA 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, Proc. Natl. Acad. Sci. USA 78:2072); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, et al., 1981, J. Mol. Biol. 150:1); and hygromycin (Santerre, et al., 1984, Gene 30:147).

10

5.4.2. ANTIBODIES TO GTIII POLYPEPTIDES

Antibodies that specifically recognize one or more epitopes of GTIII, or peptide fragments of the GTIII are also encompassed by the invention. Such antibodies include but are not limited to polyclonal antibodies, monoclonal antibodies (mAbs), humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab')₂ fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above.

The antibodies of the invention may be used, for example, in the detection of the GTIII in a biological sample and may, therefore, be utilized as part of a diagnostic or prognostic technique whereby patients may be tested for abnormal amounts of GTIII. Such antibodies may also be utilized in conjunction with, for example, compound screening schemes, as described, above, for the evaluation of the effect of test compounds on expression and/or activity of the GTIII gene product. Additionally, such antibodies can be used in conjunction with the gene therapy techniques described, below, e.g., to evaluate the normal and/or engineered GTIII-expressing cells prior to their introduction into the patient. Such antibodies may additionally be used as a method for the inhibition of abnormal GTIII activity.

For the production of antibodies, various host animals may be immunized by injection with the GTIII, or a GTIII polypeptide or peptide. In the working example described

infra, a synthetic peptide corresponding to amino acid residues 161-177 of FIG. 1 (QYKTKREEVKPPSDPEM) was used to generate monoclonal antibodies to GTIII. Such host animals may include but are not limited to rabbits, mice, hamsters and rats, to name but a few. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and Corynebacterium parvum. Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of the immunized animals.

Monoclonal antibodies, which are homogeneous populations of antibodies to a particular antigen, may be obtained by any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique of Kohler and Milstein, (1975, Nature 256:495-497; and U.S. Patent No. 4,376,110), the human B-cell hybridoma technique (Kosbor et al., 1983, Immunology Today 4:72; Cole et al., 1983, Proc. Natl. Acad. Sci. USA 80:2026-2030), and the EBV-hybridoma technique (Cole et al., 1985, Monoclonal Antibodies And Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAb of this invention may be cultivated in vitro or in vivo. Production of high titers of mAbs in vivo makes this the presently preferred method of production.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger et al., 1984, Nature, 312:604-608; Takeda et al., 1985, Nature, 314:452-454) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a

human antibody molecule of appropriate biological activity can be used. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778; Bird, 1988, Science 242:423-426; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; and Ward et al., 1989, Nature 334:544-546) can be adapted to produce single chain antibodies against GTIII gene products. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide.

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, such fragments include but are not limited to: the F(ab')₂ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab')₂ fragments. Alternatively, Fab expression libraries may be constructed (Huse et al., 1989, Science, 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

Antibodies to the GTIII can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" epitopes of the GTIII, using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, 1993, FASEB J 7(5):437-444; and Nissinoff, 1991, J. Immunol. 147(8):2429-2438).

5.5. GENE THERAPY APPROACHES

The expression of GTIII can be controlled in vivo (e.g. at the transcriptional or translational level) using gene therapy approaches to regulate GTIII activity. Such an approach may be useful to treat schizophrenia and other

GTIII-mediated disorders. Certain approaches are described below.

When an increase in the level of normal GTIII gene expression and/or GTIII gene product activity is desired, 5 GTIII nucleic acid sequences can be utilized. Where a defective GTIII gene is diagnosed, treatment can be administered, for example, in the form of gene replacement therapy. Specifically, one or more copies of a normal GTIII gene or a portion of the GTIII gene that directs the 10 production of a GTIII gene product exhibiting normal function, may be inserted into the appropriate cells within a patient or animal subject, using vectors which include, but are not limited to adenovirus, adeno-associated virus, retrovirus and herpes virus vectors, in addition to other 15 particles that introduce DNA into cells, such as liposomes.

Because the GTIII gene is expressed in the brain, e.g., the hippocampus, such gene replacement therapy techniques should be capable of delivering GTIII gene sequences to these cell types within patients. Thus, the techniques for 20 delivery of the GTIII gene sequences should be designed to readily cross the blood-brain barrier, which are well known to those of skill in the art (see, e.g., PCT application, publication No. WO89/10134, which is incorporated herein by reference in its entirety), or, alternatively, should involve 25 direct administration of such GTIII gene sequences to the site of the cells in which the GTIII gene sequences are to be expressed.

Alternatively, targeted homologous recombination can be utilized to correct the defective endogenous GTIII gene in 30 the appropriate tissue; e.g., brain tissue. In animals, targeted homologous recombination can be used to correct the defect in ES cells in order to generate offspring with a corrected trait.

Additional methods which may be utilized to increase the 35 overall level of GTIII gene expression and/or GTIII activity include the introduction of appropriate GTIII-expressing cells, preferably autologous cells, into a patient at

positions and in numbers which are sufficient to ameliorate the symptoms of schizophrenia. Such cells may be either recombinant or non-recombinant. Among the cells which can be administered to increase the overall level of GTIII gene expression in a patient are normal cells, or hippocampal cells which express the GTIII gene. The cells can be administered at the anatomical site in the brain, or as part of a tissue graft located at a different site in the body. Such cell-based gene therapy techniques are well known to those skilled in the art, see, e.g., Anderson, et al., U.S. Patent No. 5,399,349; Mulligan & Wilson, U.S. Patent No. 5,460,959.

5.6. PHARMACEUTICAL FORMULATIONS AND METHODS OF TREATMENT

The invention encompasses methods and compositions for modulating the activity of GTIII and treating schizophrenia. Because a loss of normal GTIII gene product function correlates with schizophrenia, an increase in GTIII activity would facilitate progress towards treatment.

5.6.1. DOSE DETERMINATIONS

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage

for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

5.6.2. FORMULATIONS AND USE

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients.

Thus, the compounds and their physiologically acceptable salts and solvates may be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or oral, buccal, parenteral or rectal administration.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form

of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with 5 pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and 10 preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate.

Preparations for oral administration may be suitably 15 formulated to give controlled release of the active compound.

For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use 20 according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable 25 gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g. gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as 30 lactose or starch.

The compounds may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi- 35 dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain

formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

5 The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, 10 the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or 15 hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The compositions may, if desired, be presented in a pack 20 or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

25

6. EXAMPLE: DEVELOPMENT OF MONOCLONAL ANTIBODIES
FOR HUMAN NEURON-SPECIFIC GLUTAMATE TRANSPORTER

Glutamate is a major excitatory amino acid neurotransmitter in the mammalian central nervous system.

30 The synaptic action of glutamate is thought to be terminated by a high-affinity uptake system, which is dependent on Na⁺ and K⁺. This example describes the production and characterization of a monoclonal antibody for human neuron-specific glutamate transporter.

35 A monoclonal antibody was raised against a synthetic peptide (QYTKREEVKPPSDPEM) that corresponds to amino acid residues 161-177 of the human neuron-specific glutamate

transporter cDNA. The hybridomas were screened against the free peptide by ELISA initially and the clones were selected on the basis of their immunoreactivity. Western blotting of human and rat brain synaptosomal preparations from frontal cortex revealed a immunoreactive band at ~70kD. The antibody was further characterized by immunoprecipitation and deglycosylation using N-glycosidase F. Deglycosylation of the immunoprecipitated material yielded a protein with a lower molecular weight (~65kD) on western blots. These results are consistent with the molecular weight predicted by the cloned cDNA. Immunocytochemistry of cortex, hippocampus and substantia nigra of rat brain revealed strong immunoreactivity of neuronal cell bodies. In the cortical layers, the large pyramidal neurons showed intense immunoreactivity, although there were other smaller neurons through the cortex showing less immunoreactivity. In the hippocampus neuronal somata and dendrites of the sub-fields CA1 to CA3 and the dentate gyrus were intensely immunoreactive. In substantia nigra, in addition to neuronal somata with proximal dendritic labeling, the antibody stained fine caliber fibers and numerous puncta.

7. EXAMPLE: DECREASED NEURON-SPECIFIC EXCITATORY
AMINO ACID TRANSPORTER 3 (EAAT3) IN
SCHIZOPHRENIC HIPPOCAMPUS

25

Glutamate abnormalities have been implicated in the pathophysiology of schizophrenia.

In order to evaluate alterations in glutamatergic neurotransmission, mRNA encoding the neuronal GTIII (Shashidharan et al., 1994, Brain Research 662:245-250) was measured using a quantitative nuclease protection assay in hippocampus homogenates of schizophrenics (n=19), suicides (n=15) and normal controls (n=19). A 50% decrease in GTIII mRNA was observed in schizophrenics (p=0.0005) and a 20% decrease in suicide (p=0.1227) compared to normal controls.

35

The data support a role for the hippocampus in the neuropathology of schizophrenia. The results also indicate

that at least part of this pathology may be related to decreased glutamate reuptake. Alterations in the glutamate/aspartate transporter in the schizophrenic hippocampus could reflect neuronal loss, decreased transport function resulting in rebound excess glutamate in the synapse, or hypofunction of glutamatergic neurons.

8. EXAMPLE: REDUCED LEVEL OF GTIII mRNA IN HIPPOCAMPUS OF SCHIZOPHRENICS

10 A neural circuit involving striatum/nucleus accumbens, hippocampus/parahippocampal cortex and prefrontal cortex may be involved in the pathophysiology of schizophrenia. As these regions are connected by glutamatergic neurons, a study of these neurons in the hippocampus/parahippocampal cortex
15 seemed indicated.

In situ hybridization histochemistry was performed with an [³⁵S]-labelled ribonucleotide probe for mRNA for GTIII, which has been shown to be expressed in human hippocampus (Shashidharan et al., Brain Res. 662-245, 1994), using 14 μ-
20 thick fresh frozen sections containing parahippocampal gyrus, hippocampus, presubiculum and subiculum from schizophrenics (n=9) and normal controls (n=7).

A two-way ANOVA (group by region) indicated that there was a reduction in GTIII mRNA in schizophrenics (p<.0001).
25 Post-hoc comparisons for each region indicated that this difference attained statistical significance (p≤.05) in dentate, CA1, and subiculum, where levels were 69, 67 and 61%, respectively, of normal levels. Levels were also decreased (.08≥p>.05) in CA4, presubiculum and
30 parahippocampal layers ii/iii.

These results indicate that glutamatergic hypofunction in hippocampus might contribute to the neuropathological basis of schizophrenia.

35

WHAT IS CLAIMED IS:

1. A method for identifying antipsychotic compounds comprising:
 - (a) contacting a test compound with a cell that
5 expresses a functional glutamate transporter III,
and
 - (b) determining whether the test compound modulates the
activity of the glutamate transporter III.
- 10 2. The method of Claim 1 in which the activity of the
glutamate transporter III is measured by assaying glutamate
uptake by the cell.
3. The method of Claim 1 in which the cell is a GABAergic
15 cell and the activity of the glutamate transporter is
measured by assaying GABA synthesis by the cell.
4. The method of Claim 1 in which compounds that enhance
the activity of the glutamate transporter are identified as
20 antipsychotic compounds.
5. The method of Claim 4 in which the antipsychotic
compound is useful for treating schizophrenia.
- 25 6. A method for treating schizophrenia comprising enhancing
the expression or activity of glutamate transporter III in a
patient.
7. A pharmaceutical formulation for the treatment of
30 schizophrenia, comprising a compound that enhances the
expression or activity of glutamate transporter III in a
pharmaceutically acceptable carrier.

1/4

-165 C T A G ATC CAG C A C CA TCTCT CAG CCGGT T
 TCCCGCGGCAACCGCGTCTGACGCGCCCGACTGCAGCGCGCGCTCTCAGCTCTCCCT
 CTTC T C CG CGC GC A G C T CC CGC GAGG G CACA CCGACACA AGCG G CC C T GG GCCG A G C
 -103 GTCCACCCCATCTCCCCACGTGCCGCCAGCAGCCAGTCCCGGTGCGCCACGCG—ACGCGCGACCGCGGAGCCGCGCAATAGCCCGGACAGCC
 G C A C C C C C
 1 ATG GGG AAA CCG GCG AGG AAA GGA TGC GAG TGG AAG CCC TTC CTG AAG AAT AAC TGG CTG TTG CTG TCC ACC GTG CCC-GCG GTG
 Met Gly Lys Pro Ala Arg Lys Gly Cys Glu Trp Lys Arg Phe Leu Lys Asn Asn Trp Val Leu Ser Thr Val Ala Val 28
 Asp Ser
 85 GTG CTA GGC ATT ACC ACA GCA GTC TTG GTT CGA GAA CAC AGC AAC CTC TCA ACT CTA GAG AAA TTC TAC TTT GCT TTT CCT CCA
 Val Leu Gly Ile Thr Thr Gly Val Leu Val Arg Glu His Ser Asn Leu Ser Thr Leu Glu Lys Phe Tyr Phe Ala Phe Pro Gly 56
 Val Iso Thr
 169 GAA ATT CTA ATG CCG ATG CTG AAA CTC ATC ATT TTG.CCA TTA ATT ATA TCC AGC ATG ATT ACA GGT CTT GCT GCA CTG GAT TCC
 Glu Ile Leu Met Arg Met Leu Lys Leu Ile Ile Leu Pro Leu Ile Ile Ser Ser Met Ile Thr Gly Val Ala Ala Leu Asp Ser 84
 Val Val
 253 AAC GTA TCC GGA AAA ATT CGT GTC CCC GTC GTC TAT TAT TTC TGT ACC ACT CTC ATT GGT GTT ATT CTA GGT ATT GTG CTC
 Asn Val Ser Gly Lys Ile Gly Leu Arg Val Val Val Tyr Phe Cys Thr Thr Leu Ile Ala Val Ile Leu Gly Ile Val Leu 112
 Val Leu Ile
 337 GTG GTG AGC ATC AAG CCT CGT GTC ACC CAG AAA GTG GGT GAA ATT CCG AGG ACA GGC AGC CCT GAA GTC AGT ACC GTG GAT
 Val Val Ser Ile Lys Pro Gly Val Thr Gln Lys Val Gly Glu Ile Ala Arg Thr Gly Ser Thr Pro Glu Val Ser Thr Val Asp 140
 Asp Asp
 C A C AC C A C A

FIG.1A

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421 GCC ATG TTA GAT CTC ATC AGG AAT ATG TTC CCT CAG AAT CTT GTC CAG GCC TGT TTT CAG CAG TAC AAA ACT AAC CGT GAA GAA
 Ala Met Leu Asp Leu Ile Arg Asn Met Phe Pro Glu Asn Leu Val Gln Ala Cys Phe Gln Gln Tyr Lys Thr Lys Arg Glu Glu Thr
 168

505 GTG AAC CCT CCC AGC GAT CCA CAG ATG AAC ATG ACA GAA GAG TCC TTC ACA GCT GTC ATG ACA ACT GCA ATT TCC AAC AAC AAA
 Val Lys Pro Pro Ser Asp Pro Glu Met Asn Met Thr Glu Glu Ser Phe Thr Ala Val Met Thr Thr Ala Ile Ser Lys Asn Lys
 Thr Ala Ser Asp Thr Gly Lys Gly Val
 196

589 ACA AAG GAA TAC AAA ATT GTT GGC ATG TAT ICA GAT GGC ATA AAC GTC CTC GGC TTG ATT GTC TTT TCC CTT GTC TTT GGA CTT
 Thr Lys Glu Tyr Lys Ile Val Gly Met Tyr Ser Asp Gly Ile Asn Val Leu Gly Leu Ile Val Phe Cys Leu Val Phe Gly Leu
 Arg Val Leu
 224

673 GTC ATT GCA AAA ATG CGA GAA AAG GGA CAA ATT CTG CTG GAT TTC TTC AAT GCT TTG AGT GAT GCA ACC ATG AAA ATC GTT CAG
 Val Ile Gly Lys Met Gly Glu Lys Gly Gly Gln Ile Leu Val Asp Phe Phe Asn Ala Leu Ser Asp Ala Thr Met Lys Ile Val Gly
 252

757 ATC ATC ATG TGT TAT ATG CCA CTA GGT ATT TTG TTC CTG ATT GCT GGG AAG ATC ATA GAA GTT GAA GAC TGG GAA ATA TTC GCA
 Ile Ile Met Cys Tyr Met Pro Leu Gly Ile Leu Phe Leu Ile Ala Gly Lys Ile Ile Glu Val Glu Asp Trp Glu Ile Phe Ala
 Arg
 280

841 AAG CTG CCC CTT TAC ATG GCC ACA GTC CTG ACT GGC CTT GCA ATC CAC TCC ATT GTA ATT CTC CCG CTG ATA TAT TTC ATA GTC
 Lys Leu Gly Leu Tyr Met Ala Thr Val Leu Thr Gly Leu Ala Ile His Ser Ile Val Ile Leu Pro Leu Ile Tyr Phe Ile Val
 Val Ser
 308

FIG.1B

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925	GTA CGA AAC CCT TTC CGA TTT GCC ATG GGA ATG GCC CAG GCT CTC CTG ACA GCT CTC ATG ATC TCT TCC AGT TCA GCA ACA Val Arg Lys Asn Pro Phe Arg Phe Ala Met Gly Met Ala Gln Ala Leu Leu Thr Ala Leu Met Ile Ser Ser Ser Ala Thr	336
1009	CTG CCT GTC ACC TTC CGC TGT GCT GAA GAA AAT AAC CAG GTC CAC AAG AGG ATC ACT CGA TTC GTG TTA CCC GTT CGT GCA ACA Ile Pro Val Thr Phe Arg Cys Ala Glu Glu Asn Asn Gln Val Asp Lys Arg Ile Thr Arg Phe Val Leu Pro Val Gly Ala Thr	364
1093	ATC AAC ATG GAT GCG ACT GCG CTC TAT GAA GCA GTC GCA GCG GTG TTT ATT CCA CAG TTG AAT GAC CTG GAC TTG GCG ATT GCG Ile Asn Met Asp Gly Thr Ala Leu Tyr Glu Ala Val Ala Ala Val Phe Ile Ala Gln Leu Asn Asp Leu Asp Gly Ile Gly	392
1177	CAG ATC ATC ACC ATC AGT ATC ACC GCG ACA TCT GCC AGC ATC GGA GCT GCT CCC GTG CCC CAG GCT GCG CTG GTC ACC ATG GTG Gln Ile Thr Ile Ser Ile Thr Ala Thr Ser Ala Ser Ile Gly Ala Ala Gly Val Pro Gln Ala Gly Leu Val Thr Met Val	420
1261	ATT GTG CTG AGT GCG GTG GCG CTC GCG CAG-GAT CTC ACC CTG ATC ATT GCT GTC GAC TGG CTC CTG GAC CCG TTC AGG ACC Ile Val Leu Ser Ala Val Gly Leu Pro Ala Glu Asp Val Thr Leu Ile Ile Ala Val Asp Trp Leu Leu Asp Arg Phe Arg Thr	448
1345	ATG GTC AAC GTC CTT GGT GAT GCT TTT GCG ACG GGC ATT GTG GAA AAG CTC TCC AAG AAG GAG CTG GAG CAG ATG GAT GTT TCA Met Val Asn Val Leu Gly Asp Ala Phe Gly Thr Gly Ile Val Glu Lys Leu Ser Lys Lys Glu Leu Glu Gln Met Asp Val Ser	476
1429	TCT GAA GTC AAC ATT GTG AAT CCT TTT CCC TTG GAA TCC ACA ATC CTT GAC AAC GAA CAC TCA GAC ACC AAG AAG TCT TAT GTC Ser Glu Val Asn Ile Val Asn Pro Phe Ala Leu Glu Ser Thr Ile Leu Asp Asn Glu Asp Ser Thr Lys Lys Ser Tyr Val Iso	304

FIG. 1C

4/4

C A A T G G A G G C T T T G C A G T A G A C A A G I C T G A C A C C A T C T C A T T C A C C A G A C C A T T A A T T G A T G A T A T A C A G A C C T C C A G A T T A T T T C T A T
 Asn Gly Gly Phe Ala Val Asp Lys Ser Asp Thr Ile Ser Phe Thr Gln Thr Ser Gln Phe *
 524
 C C T T C A G A G A C T C A T C T A G T G A A A C A T T A A T T G A T G A T A T A C A G A C C T C C A G A T T A T T T C T A T
 1605 ' S G A A G G A C A T T T C C G T G A G A G T C A T C T C A A A C A C T G C T T A A C C A A A G A G A A A C A C T A A T C C C C A A G T C T A C A T T T G A T A T A C A G A C C T C C A G A T T A T T T C T A T
 C A A G C C G T C G A C C C T T T T C C C C T G G T T T C C C A T T C C C G T A A G T T G A A C C A C T A A T T A A G G A A A G
 1715 A T T T G C A T T C T G C A C C C T T T T C C C C T G G T T T C C C A T T C C C G T A A G T T G A A C C A C T A A T T A A G G A A A G

FIG. 1D

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/16219

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12N 15/12; G01N 33/53

US CL : 536/23.5; 435/7.1, 7.2, 7.21, 69.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.5; 435/7.1, 7.2, 7.21, 69.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS and DIALOG (MEDLINE, BIOSIS, WORLD PATENT INDEX) search terms: glutamate, receptor, agonist, antagonist, schizophrenia, psychotic, antipsychotic, GABA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KANAI et al. Primary structure and functional characterization of a high-affinity glutamate transporter. Nature. 03 December 1992, Vol. 360, No. 6403, pages 467-471, see entire document.	1-5
Y	SQUIRES et al. A review of evidence for GABergic predominance/glutamatergic deficit as a common etiological factor in both schizophrenia and affective psychoses: More support for a continuum hypothesis of "functional" psychosis. Neurochemical Research. October 1991, Vol. 16, No. 10, pages 1099-1111, see entire document.	1-5



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
B earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

date of the actual completion of the international search

02 DECEMBER 1997

Date of mailing of the international search report

23 DEC 1997

Name and mailing address of the ISA/US
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/16219

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SHASHIDHARAN et al. Neuron-specific human glutamate transporter: Molecular cloning, characterization and expression in human brain. Brain Research. 1994, Vol. 662, No. 1-2, pages 245-250, especially Figure 1.	1-5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/16219

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-5

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/16219

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-5, drawn to a method for identifying antipsychotic compositions.

Group II, claim 6, drawn to a method for treating schizophrenia.

Group III, claim 7, drawn to a pharmaceutical composition for treating schizophrenia.

The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The special technical feature of the first appearing invention, Group I, is the steps of contacting the glutamate III receptor with a test compound and determining the change in receptor activity. These features are not present in Groups II and III.